DETAILED ACTION

1. It is noted that instant application is being prosecuted by a different Examiner whose name appears at the end.

Claim Status

2. Amendment filed on December 18, 2007 is acknowledged. Applicant has amended claims 1, 4-6, 8, and 10; added new claims 11 and 12. Consequently pending amended claims 1-12 will be examined in this action.

Response to Claim Objections

3. Amendment to claims 8 and 10 overcome the objections raised in office action mailed on September 18, 2007.

Response to Claim Rejections - 35 USC § 112

4. Amendments to claims 1, 3, 4-6, overcome the 112 2nd rejections of claims 1-9 raised in the Office Action mailed on September 18, 2007.

Response to Arguments re Claims Rejected Under 103

5. Applicant's arguments with respect to claims1-4, and 6-9 rejected over Uematsu et al. in view of Kato and Kaufman et al. as evidenced by Ronaghi et al., have been considered but are moot in view of the new ground(s) of rejection. Applicant has amended the base claim which necessitated the new grounds of rejection.

6. Rejection of claims 1-9 over Uematsu et al. in view of Kwon et al., and Kaufman et al. as evidenced by Ronaghi et al., are also not valid in view of the amendment. Accordingly these rejections are withdrawn. The new amended claims are being rejected using new grounds of rejection.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 1-12 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Regarding claim 1, amendment introduces new limitations:

(c) "quantitatively detecting" a sequence of amplified DNA---. Examiner found no support in the specification as filed for "quantitatively detecting a sequence". The methods of detection taught for detection do show simultaneous detection of multiple genes but all the measurements are **relative expression levels**. "quantitatively detecting" a sequence is not taught. This is a NEW MATTER REJECTION.

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in *Ex parte Wu*, 10 USPQ2d 2031, 2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is

followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required feature of the claims. Note also, for example, the decisions of *Ex parte Steigewald*, 131 USPQ 74 (Bd. App. 1961); *Ex parte Hall*, 83 USPQ 38 (Bd. App. 1948); and *Ex parte Hasche*, 86 USPQ 481 (Bd. App. 1949).

In the present instance, claim 1 recites the broad recitation ----mixture by bioluminescence analysis", and the claim also recites in the next phrase "preferably pyrosequencing" which is the narrower statement of the range/limitation. Hence claims 1-11 are rejected as being indefinite.

Similarly claim 10 recites the broad recitation ----"a single stranded binding protein", and the claim also recites in the next phrase "(e.g. SSB)" which is the narrower statement of the range/limitation. Hence claim 10 is rejected as being indefinite as it is not clear whether applicant wishes to claim the broad or the narrow limitation.

Appropriate correction is required.

Claim Interpretation

10. In view of the 112 2nd issue raised above. For applying art Examiner is interpreting claim 1 to mean that applicant actually wishes to claim narrow limitation that was introduced in the present amendment namely pyrosequencing instead of the broad limitation bioluminescence. For claim 10 Examiner is interpreting the claim broadly and thinks applicant wants to claim a single stranded binding protein and not just be limited to SSB.

Claim Rejections - 35 USC § 103

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 1-4, and 6-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uematsu et al. (US 6,225,064) in view Kaufman et al. (US 6,383,754) and Ahmadian et al. (2000) Analytical Biochem. 280: 103-110 (provided by applicant in the IDS).

Regarding claim 1, Uematsu et al. teach:

A method for comparing gene expression level (see Uematsu et al. col. 1 line 35 and col. 2 lines 4-5 where expression profile of a gene and expression level of each expressed gene determination is taught thus the method teaches a method for comparing gene expression level) comprising the following steps includes:

(a) labeling mRNA (see col. 1 line 30-31 where cDNA or mRNA template is taught and where use of plural primers is taught for labeling the cDNA or mRNA)

from different sources (see Example 1 col. 6 where RNA isolated from different sources liver and kidney is taught to make cDNA),

with DNA fragments having different base orders, (see col. 3 lines 17-20 where DNA fragment having different base order are composed of same modules arranged in different orders. Each module is composed of 4 to 6 nucleotide. Thus by teaching these fragments shown in Fig, 2 DNA fragments having different base orders is taught. Here both the modules labeled I through IV taught are DNA fragments having different base orders as well as the FA and FB shown

exemplify DNA fragments having different base orders, also see col. 3 lines 57-61 step I. where ligating the primers made of modules is taught as a method of labeling the DNA)

and mixing the labeled molecules equally to obtain a template for polymerase chain reaction (PCR) (see step 2 col. 3 lines 62-53) ;

(b) performing a polymerase chain reaction using source-specific primers with different sequences (see step a above where Uematsu et al. teach modules I through IV as DNA fragment having different base order. These modules have different sequences is taught in col. 6 line 45 where 4 modules of 4 nt each having different base orders is taught. Therefore the primers that are complementary to these source specific primers ie. modules will necessarily have different sequences)

and gene-specific primers (see step 3 col. 3 lines 64-67. Prior art is using terminology "primers complementary to oligomer" which is equivalent of term source specific primer of instant invention and "primers corresponding to the individual group" in prior art is equivalent of "gene-specific primers" of instant invention); and

(c) quantitatively (see col. 3 line 53 where quantitative comparison of the ratio of DNA fragments present in plural samples is taught. Thus teaching quantitative detection method) detecting a sequence of amplified DNA (Uematsu et al. teach detecting a sequence of amplified DNA by using electropherogram analysis)

Regarding claim 2, Uematsu et al. teach wherein the mRNA from different sources is an expressed mRNA of a given gene from different individuals of a species, or is an expressed mRNA of a given gene from different organs of an individual, or is an expressed mRNA of a given gene of a same species at different states of chemical stimulation or physical stimulation

(See Example 1 col. 6 where mRNA isolated from different sources liver and kidney ie. different organs of an individual is taught to make cDNA) .

Regarding claim 3, Uematsu et al. teach wherein each of the source-specific primers includes an identical base species and base number but a different base order, each primer representing a gene source.(see col. 3 lines 33-35 where nucleotide composition of each of the primers is taught as never changed because only the order of modules is different in each primer, but each primer is made of same modules. Thus teaching each of the source-specific primers includes an identical base species and base number but a different base order, each primer representing a gene source).

Note added by Examiner: Primers of Uematsu et al. are composed of many modules and each module having 4 to 6 nucleotide. So the total number of bases of a given nucleotide say A, C, G or T in the primer stay constant because the total no of modules is identical and composition of each module is specified. In view of this teaching one of ordinary skill can logically understand that if total number of nucleotides of a given nucleotide ie. dNTP in the primer are kept constant, while varying the order to produce a set of different primers that can be used to label different source specific RNAs then this set of primers will meet the above limitation namely wherein each of the source-specific primers includes an identical base species and base number but a different base order).

Regarding claim 4, Uematsu et al. teach wherein labeling mRNA from different sources with DNA fragments having different base order includes methods for the labeling: a first of the method including:

performing a reverse transcription-polymerase chain reaction (RT -PCR) to obtain complementary DNA(cDNA) fragments of a given gene in each source (see Example I col. 6 lines 15-16); digesting cDNA into fragments with a suitable length using a restriction endonuclease (see col 6 lines 16-29 where digestion with restriction endonuclease is taught) and ligating each of the digested cDNA fragments with a selective adapter (see Fig. 1 where ligation with oligo 11 and oligo 12 is taught), a different adapter corresponding to mRNA from a different source (here diff adapters oligo 11 and oligo 12 are ligated to two different populations of DNA digested by restriction enzymes. These cDNA are obtained from rat liver and rat kidney RNAs (see col. 6 lines 7-8 thus teaching adapter oligo 11 and oligo 12 different adapter corresponding to mRNA from a different source).

Regarding claim 6, Uematsu et al. teach wherein the DNA fragments include identical base species and base number but different base order, each of the fragments having a same melting temperature (see 3 above where Examiner has described in detail DNA fragments include identical base species and base number but different base order. If the number of nucleotides of a given type is fixed only there order is different the inherently each of the DNA fragments will have same melting temperature. This is also taught explicitly by Uematsu et al. in col. 5 lines 10-11 where they state "plural primers of plural primer sets are of the same melting temperature" thus teaching DNA fragments having a same melting temperature.

Regarding claim 11, Uematsu et al. teach wherein labeling mRNA from different sources (see abstract where detection of plural sample is taught) with DNA fragments having different base orders includes labeling mRNA from six kinds of sources with DNA fragments having six kinds of different base orders (see above for claim 1 where modules having 4 different

nucleotides is taught. 4 nucleotides a, c, g, t can be permuted in different orders to yield (4x4=16) a total of 16 sequences. That will have identical nucleotide content but different base orders. So 6 of these permutations can be used to label 6 different RNAs (plural) thus labeling mRNA from different sources with DNA fragments having different base orders includes labeling mRNA from six kinds of sources with DNA fragments having six kinds of different base orders).

Regarding claim 12, Uematsu et al. teach wherein the six kinds of different base orders include “cgat", “gcat", “agct", “gact", “cagt" and "acgt", only three kinds of dNTPs, including dTTP, dGTP, and dCTP, are used in the bioluminescence analysis (see as explained for claim 11 above Uematsu et al. teach 4x4=16 combinations of the 4 nucleotides. This set includes the subset of 6 permutations claimed in the instant claim. If only these 6 were used to label the 6 different RNA populations then it’s clear that last base t is common to all the tags. Hence the tags differ by only the order of appearance of first 3 bases namely c, g or a. So the three dNTP's which are complementary to these will be dGTP, dCTP and dTTP. In other words a mixture of the above 3 dNTPs and the order in which they are added during pyrosequencing (bioluminescence analysis) will be inherently sufficient to determine the type and amount of the labeled RNA species present.

Regarding claim 1, Uematsu et al. do not teach:

detecting a sequence of amplified DNA mixture by bioluminescence analysis, preferably pyrosequencing, the base type in the sequencing profile, the signal intensity of the corresponding base representing the gene source and a relative expression level at the source.

Regarding claim 1, Kaufman teaches:

quantitatively detecting a sequence of amplified DNA mixture by bioluminescence analysis, preferably pyrosequencing, the base type in the sequencing profile, the signal intensity of the corresponding base representing the gene source and a relative expression level at the source (See Kaufman col.53 lines 4-13 where pyrosequencing is taught as a method of detecting a sequence of amplified DNA mixture by bioluminescence analysis. Kaufman et al. also teaches, expression level of a given tag (the source specific primer of instant invention) is proportional to the number of times the sequence occurs. Thus Kaufman et al. teach quantitative detection and also teaching the signal intensity of the corresponding base representing the gene source and a relative expression level at the source (when you compare the number of times the sequence from one source is detected vs. the number of times the sequence from another source is detected—this gives the relative expression level at the source).

Regarding claim 7, Kaufman et al. teach wherein the bioluminometric assay is based on a quantitative determination of pyrophosphate released from an extension reaction (see col. 53 line 7 where pyrosequencing is taught as a method to detect the sequence of the unknown base. By teaching pyrosequencing Kaufman et al. teach wherein the bioluminometric assay is based on a quantitative determination of pyrophosphate released from an extension reaction.

Regarding claim 8, Kaufman et al. teach wherein the extension reaction is polymerization of single-stranded PCR products annealed with a given primer or primer mixtures by DNA polymerase when a deoxynucleotide (dNTP) added in a given order, or a dideoxynucleotide (ddNTP) added in a given order, or an analog of dNTP or ddNTP added in a given order is complementary to the template (see col. 51 lines 54 through -col. 53 line 13 where details of pyrosequencing are taught. Especially see col. 53 line 3 where retention of single-stranded PCR

products called amplicon is taught for further step of pyrosequencing. Details of how pyrosequencing is conducted is known to one of ordinary skill and is evidenced in detail by Ahmadian et al. (provided by applicant in the IDS).(See Ahmadian et al. page 104 section Pyrosequencing where extension reaction is polymerization of single-stranded PCR products annealed with a given primer or primer mixtures by DNA polymerase when a deoxynucleotide (dNTP) added in a given order is taught. This extension reaction is inherently is complementary to the template. That is how the sequence id determined (see Fig. 4) (also see Ahmadian et al. page 108 par. 3 under discussion where sequential nucleotide addition in a specific dispensing order based on the sequence to be detected (SNP in the case of prior art) is taught.

Regarding claim 9, Kaufman et al. teach wherein the single-stranded PCR products are obtained by treating the PCR products of claim 1 with a physical method or a chemical method, the physical method being to use a biotinylated primer for PCR amplification and then to prepare single-stranded DNAs (see col. 53 lines 1-3 where biotinylated primer for PCR amplification is taught and use of biotin to retain single-stranded DNAs is taught) by a solid phase method (see col. 21 lines 47-56 where solid phase methods of capturing sample fragments are taught and col. 21 lines 57-7 where various solid phase substrates suitable for use are taught).

Regarding claim 10, Kaufman et al. teach method of claim 7 namely Pyrosequencing. The details of how this is conducted are provided by Ahmadian et al. wherein the extension reaction is polymerization of the PCR products treated by enzymes to degrade PPi produced during PCR reaction, excess dNTPs and excess primers, a single-strand binding protein (e.g. SSB) being added into the treated PCR products (see Ahmadian et al. Fig. 1 where principle of pyropsequencing is taught. Here DNA polymerase releases PPi during extension. Enzyme ATP

sulfurylase converts the PPi to produce ATP. Enzyme luciferase uses ATP to generate detectable light (bioluminescence). Enzyme apyrase degrades excess added nucleotide or excess primers (Note Ahmadian et al. teaches a situation where sequencing primer 100nm is annealed to single stranded DNA. This annealed substrate was added to the pyrosequencing mix without removal of excess unhybridized primer (see page end of 104 par. 1). The apyrase enzyme taught will remove both excess added nucleotide or excess primers. The protein streptavidin taught by Ahmadian et al. used to prepare streptavidin –coated superparamagnetic beads (see page 105—pyrosequencing par. 1) is a single-strand binding protein that is added to treated PCR products.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to practice the method of Kaufman et al. in the method of Uematsu et al. The motivation to do so is provided by both Kaufman et al. and Ahmadian et al.

Kaufman et al. teach comparison of gene expression pattern (see col. 1 lines 19-20). They also teach use of quantitative measurement to determine the amount or intensity of a label (see col. 21 lines 1-2). They also teach use of pyrosequencing to compare gene expression.

Ahmadian et al. state "pyrosequencing, that allows for rapid real-time determination of 20-30 base pairs of a target sequence.------pyrosequencing is performed in an automated microtiter –based pyrosequencer instrument, which allows simultaneous analysis of 96 samples within 10 minutes. Each round of nucleotide dispensing takes approximately 1 min and thus offers a rapid way to determine the exact sequence of the ---- including adjacent positions as a control" (see page 108 par. 1 discussion). They go on to state " the advantage of using cyclic addition of nucleotides is that it generates a complete pattern difference along the sequence of the three variants of the SNP. In contrast, the sequential nucleotide addition (i.e. SNP-specific

dispensing order) generates differences in three peak positions and can then be designed so that the individual allele extensions are in phase. -----Another advantage of using sequential nucleotide addition is that the pyrosequencing will be finished twice as fast compared to the cyclic protocol" (see page 108 par. 3).

Thus the above teachings explicitly tell one of ordinary skill the advantages of using sequential nucleotide addition in pyrosequencing for comparing the gene expression levels.

13. Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over Uematsu et al. (US 6,225,064) in view Kaufman et al. (US 6,383,754) and Ahmadian et al. (2000) as applied to claim 4 above further in view of Kwon et al., "Efficient amplification of multiple transposon-flanking sequences," J. Microbiol. Methods, 2000, Vol.41, pp.195-199.

Regarding claim 5, Uematsu et al. in view Kaufman et al. and Ahmadian et al. teach method of claim 4, but they do not teach wherein the selective adapter is a cuneal dsDNA (double strand DNA) containing a part of sequences complementary to recognition sequences of the restriction endonuclease and can be fully ligated with restriction enzyme cutting ends in DNA fragment by a DNA ligase, a 5' terminal region of one of the strands in the adapter containing an arbitrary sequence, used to represent the particular gene source it's ligating to, a 3' terminal region of the other strand in the adapter containing bases non-complementary to a opposite strand, or a 3' end of the other strand in the adapter being modified to block ability of extension reaction by DNA polymerase, and the adapter having a structure of a "Y" shape consisting of two strands, one end of the adapter being divided into two branches due to no complementary bases, and the other end being formed of a shape of restriction enzyme cutting site.

Regarding claim 5, Kwon et al. teach wherein the selective adapter is a cuneal dsDNA (double strand DNA) containing a part of sequences complementary to recognition sequences of the restriction endonuclease and can be fully ligated with restriction enzyme cutting ends in DNA fragment by a DNA ligase, a 5' terminal region of one of the strands in the adapter containing an arbitrary sequence, used to represent the particular gene source it's ligating to, (i.e. sequences specific to gene sources) a 3' terminal region of the other strand in the adapter containing bases non-complementary to a opposite strand, or a 3' end of the other strand in the adapter being modified to block ability of extension reaction by DNA polymerase, and the adapter having a structure of a "Y" shape consisting of two strands, one end of the adapter being divided into two branches due to no complementary bases, and the other end being formed of a shape of restriction enzyme cutting site (see abstract Fig.1, pp.197, and pp.198, 1st column where details of Y linker are taught). The ligated linker DNA fragment is then subjected to PCR using a primer targeting the transposon (i.e. gene-specific primer) and a Y linker primer (i.e. a source-specific primer).

It would have been prima facie obvious to one of ordinary skill in the art to practice the method of Kwon et al. in the method of Uematsu et al.; Kaufman et al. and Ahmadian et al. at the time the invention was made. The motivation to do so is provided by Kwon et al.

Kwon et al. teaches that it was conventional in the art at the time of the invention to conduct a reaction involving ligation of selective Y shaped adaptors to fragmented DNA, and subsequently amplifying the ligated fragments using a gene-specific primer along with a primer specific for the ligated adaptors. In page 198 par. 1 Kwon et al. explain how the presence of the non complementary region on the 5' end of Y linker prevents the extension of DNA leading to

selective amplification under stringent conditions. Kwon et al states that the method has "general application for the specific amplification of the sequences that flank the known region," and provides a method for analyzing multiple sequences for sequence variations (pp.198, last paragraph).

Therefore, the skilled artisan would have had a reasonable expectation of success in using a gene-specific primer along with the source-specific primers, adaptors which are Y shaped, and bioluminescence analysis therein resulting in a very selective, fast and rapid, thereby a cheaper protocol that is capable of handling multiple simultaneously for comparing gene expression level.

Conclusion

14. All claims under consideration 1-11 are rejected over prior art.

15. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUCHIRA PANDE whose telephone number is (571)272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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